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Fatty acid characteristics in two symbiotic gastropods from a deep hydrothermal vent of the West Pacific

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ABSTRACT: Two deep-sea gastropods, *Ifremeria nautilei* and *Alviniconcha hessleri*, collected on a hydrothermal site of the North Fiji Basin (Southwestern Pacific) were analysed for polar and neutral lipids using gas-liquid chromatography/mass spectrometry. A high level of monounsaturated fatty acids (MUFAs) and a low level of ω3 series polyunsaturated fatty acids (PUFAs) indicated that nutrition of both gastropods was related to a food web based mainly on bacterial supply. From differences in MUFA distribution between the 2 gastropods, it appeared that most of the energy requirements of *A. hessleri* were supplied by sulfur-oxidizing endobacteria whereas *I. nautilei* probably had a mixotrophic diet based on endogenous as well as exogenous bacteria. Given the relatively high level of linoleic acid, which represented from 2 to 8% of the phospholipid fatty acids, hydrothermal gastropods did not appear to be depleted in ω6 PUFAs. It was hypothesized that they obtain linoleic acid from a pathway different to that in heterotrophic marine molluscs. In contrast to ω6 PUFAs, both hydrothermal gastropods appeared to be depleted in ω3 PUFAs, indicating the limited importance of photosynthesis-based food supplies. Some non-methylene-interrupted dienes, particularly 20:2ω9,15 which represented from 9 to 18% of the phospholipid fatty acids, may be synthesized by deep-sea symbiotic molluscs in order to restore the depleted ω3 PUFAs considered as essential for animals. Gills of both gastropods had high levels of neutral lipids, mainly MUFAs that may have originated from degradation of endobacterial phospholipids.

KEY WORDS: Hydrothermal vents · Gastropods · Chemosynthetic bacteria · Biomarkers · Fatty acids · Phospholipids · Neutral lipids

INTRODUCTION

Communities associated with deep hydrothermal vents from the East Pacific are usually dominated, in terms of biomass, by a small number of invertebrates belonging to 2 main taxa, namely vestimentiferan tubeworms and bivalve molluscs (Hessler & Smithey 1983, Tunnicliffe 1991, Childress & Fisher 1992). The recently discovered hydrothermal vents situated on spreading axes of back-arc basins in the Western Pacific display radically different biological assemblages that are characterized by the dominance of gastropods as primary consumers (Jollivet et al. 1989, Bouchet & Waren 1991, Waren & Bouchet 1993). In particular, 2 large species belonging to the family Provannidae, *Alviniconcha hessleri* (Okutani & Ohta 1988) and *Ifremeria nautilei* (Bouchet & Waren 1991), closely associated with the fluid emissions, compose most of the vent biomass (Bouchet & Waren 1991, Galkin 1992, Desbruyères et al. 1994). Electron microscopic and enzymatic studies have shown that these provannid gastropods harbour chemoautotrophic bacteria in specialized gill cells (Stein et al. 1988, Endow &

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Ohta 1989, Galkin 1992, Desbruyères et al. 1994). The symbionts appear to be sulfur-oxidizing Gram-negative bacteria but another type, probably related to nitrifying or methylotroph bacteria, may coexist in the same vacuoles (Endow & Ohta 1989, Galkin 1992). *A. hessleri* and *I. nautilei* are the only gastropods presently known to be associated with chemosynthetic endobacteria and their ecological status, particularly with respect to their nutritional strategy, and specific metabolic characteristics are little known. Although their radula and gut are reduced, their stomach contains mineral and animal fragments. This suggests that they have a mixotrophic diet supported both by endobacterial carbon transfer and grazing (Stein et al. 1988, Bouchet & Waren 1991, Desbruyères et al. 1994).

Bivalve molluscs associated with chemolithoautotrophic bacteria, such as lucinids, solemyids and thyasirids from littoral reduced sediments (Conway & McDowell Capuzzo 1990, 1991, Conway et al. 1992, Zhukova et al. 1992, Fullarton et al. 1995, Pranal 1995) and mytilids and vesicomyids from deep-sea hydrothermal vents (Ben-Mlih et al. 1992, Pranal 1995, Pranal et al. in press), have been analyzed using fatty acids as biomarkers. These studies helped to characterize the metabolism and the ecology of these associations. We have now applied the same approach to the 2 hydrothermal gastropods, *Ifremeria nautilei* and *Alvinicancha hessleri*.

MATERIALS AND METHODS

Station location and description. Specimens of *Ifremeria nautilei* and *Alviniconcha hessleri* were collected at the hydrothermal vent site 'Kayo' (16° 59' S, 173° 43' W; 1977 m) in the North Fiji back-arc basin (Southwestern Pacific) during the RV 'Yokosuka' cruise (1991). 'Kayo' is located on a very active vent field lying on a steep slope. A 2 m high chimney stood at its center, emitting transparent fluid with a high temperature (296°C), strong H_2S concentration (4.0 mmol kg^{-1}) and low pH of the end member fluids, about 4.7 (Ishibashi et al. 1994). Methane is present at concentrations of 45 µmol kg^{-1} (D. Grimaud pers. comm.). Basalts lying on this site are covered by scattered bacterial mats. The biological community is similar, but with more abundant individuals, to that of the 'White Lady' site described in Desbruyères et al. (1994). Gastropods, especially *Ifremeria nautilei* and *Alviniconcha hessleri*, are the dominant organisms. The associated fauna comprises essentially symbiotic mussels belonging to the species *Bathymodiolus brevior* (Von Cosel et al. 1994).

Lipid analysis. For both gastropod species, tissues were analyzed from 3 different specimens collected during the same dive, in the same clump of animals. Gills and mantles were dissected immediately after arrival on board. Samples were stored in liquid nitrogen and freeze-dried in the laboratory.

Lipid extraction, fatty acid purification, and quantification by capillary gas chromatography (GC) were performed as previously described (Bligh & Dyer 1959, White et al. 1977). Essentially, a modified Bligh & Dyer chloroform-methanol lipid extraction was used. Total extractable lipids were fractionated by silicic acid column chromatography. The fatty acid esters linked to the phospholipids and the triglycerides were methylated by acidic methanolysis of the polar and neutral lipid fractions, respectively. The resulting fatty acid methyl esters were purified by thin-layer chromatography before GC analysis. Quantification was based on comparison of peak areas to an internal injection standard (19:0). Tentative peak identifications were based on co-elution on a 50 m nonpolar fused silica capillary column (CP-Sil-5CB, 0.25 mm i.d., 0.25 µm film thickness; Chrompack, Middelburg, The Netherlands) with standards obtained from either Sulpeco, Inc. (Bellefonte, PA, USA), identified laboratory standards or previously analysed lipid extracts from *Mytilus galloprovincialis* and *Bathymodiolus brevior* (Pranal 1995, Pranal et al. in press).

Fatty acid structural verification. Identification of fatty acid methyl esters, position of methyl branching, and degree of unsaturation were verified by GC/mass spectrometry (MS) as described previously (Nichols et al. 1989, Guezennec 1991). Monounsaturation position and geometry were chemically determined by using dimethyl disulfide (Aldrich Chemical Co., Inc., Milwaukee, WI, USA) and 1 drop of iodine solution (60 mg of iodine in 1 ml of diethyl ether). Reactions were performed in 2 ml glass vials sealed with a Teflon-lined septum at 50°C for 48 h, cooled, and diluted with 200 µl of hexane. The iodine was removed by shaking with 200 µl of 5% $Na_2S_2O_3$. The upper organic phase was removed, the aqueous phase was washed with 100 µl of hexane, and the combined hexane fractions were dried under nitrogen gas before GC and GC/MS analysis. Detection of both the omega (ω) and delta (Δ) fragments confirmed the original double-bond position of the monounsaturates (Guezennec 1991). Geometry was verified by GC separation of the dimethyl disulfide adducts of identical positional isomers analysed by MS.

Fatty acid nomenclature. Fatty acids are characterized by chain length. Carbon number is followed by a colon, then the number of double bonds. The position of unsaturation is specified from the methyl end (ω) or from the carboxyl end (Δ). The prefixes 'i' and 'a' refer to iso and anteiso branching, respectively. The suffix 'x' refers to undetermined number of double bonds

and the suffixes 'a', 'b' and 'd' to undetermined positions of unsaturations.

RESULTS

Fatty acid composition of polar lipids

Fatty acid concentrations of the polar lipids (Table 1) were quite similar for both gastropods. Higher concentrations were found in gills (12.9 and 9.7 mg g^{-1} dry weight for *Ifremeria nautilei* and *Alviniconcha hessleri*, respectively). Values in mantles were 3 to 4.5 times less (about 3 mg g^{-1} dry weight). The preponderant fatty acids in gills and mantles of both species were the saturated fatty acid 16:0, the monounsaturated fatty acids (MUFAs), 16:1ω7 and 18:1ω7, and the non-methylene-interrupted dienoic (NMID) fatty acid 20:2ω9,15. Hydrothermal gastropods are characterized by a high level and diversity of MUFAs. In *A. hessleri* tissues, most of them correspond to even-numbered fatty acids having a double bond in the ω7 or ω9 position. The total of these MUFAs represented 49 and 34% of the fatty acids in gills and in mantles, respectively (Table 2). Despite the high level of ω7 and ω9 series MUFAs in *I. nautilei* tissues (26 and 28% of the total fatty acids in gills and mantles, respectively), this gastropod clearly differed from *A. hessleri*, displaying relatively high levels of even-numbered MUFAs with a double bond in the ω6 or ω8 position, which constitute about 7% of the total fatty acids. This difference was especially pronounced with respect to ω8 MUFAs, which were exclusively found in *I. nautilei* tissues. Percentages of ramified fatty acids were low, and were lower in *A. hessleri* tissues (less than 0.5% of the total fatty acids) than in *I. nautilei* tissue (1.2 and 3.1% of the fatty acids in gills and mantles, respectively). According to the species and the organ, the sum of the polyunsaturated fatty acids (PUFAs) varied from 41 to 43% of the total fatty acids, except for the gills of *A. hessleri*, in which this sum was much lower (27% of the total fatty acids). PUFAs having a double bond in the ω3 or ω6 position composed from 1/3 to 1/2 of the Σ PUFAs and mainly consisted of 18:2ω6, 20:4ω6, 20:5ω3 and 22:5ω3 for *I. nautilei*, and 18:2ω6, 20:4ω6 and 20:5ω3 for *A. hessleri*.

Table 1. Fatty acid composition of the polar lipids, as a percentage of total fatty acids, from the symbiotic gastropods *Ifremeria nautilei* and *Alviniconcha hessleri* collected at the hydrothermal vent site Kayo (North Fiji Basin). Values represent the mean of 3 different animals ± SD. tr: trace

Fatty acid	*Ifremeria nautilei*		*Alviniconcha hessleri*	
	Gills	Mantles	Gills	Mantles
14:0	0.77 ± 0.30	1.14 ± 0.49	0.56 ± 0.08	1.56 ± 0.65
15:0	0.05 ± 0.01	– –	0.19 ± 0.07	0.14 ± 0.04
16:0	10.91 ± 0.66	11.11 ± 0.98	12.43 ± 0.80	11.43 ± 0.38
17:0	0.26 ± 0.04	0.32 ± 0.08	0.41 ± 0.13	0.40 ± 0.13
18:0	4.20 ± 0.53	3.77 ± 0.65	3.31 ± 0.73	4.30 ± 1.04
a 15:0	0.54 ± 0.05	– –	– –	– –
i 17:0	0.32 ± 0.17	0.42 ± 0.06	0.19 ± 0.21	0.20 ± 0.03
a 17:0	– –	– –	tr –	– –
i 19:0	0.19 ± 0.02	0.36 ± 0.16	0.27 ± 0.10	– –
a 19:0	0.12 ± 0.02	1.31 ± 0.57	– –	– –
16:1ω5	0.38 ± 0.17	0.35 ± 0.14	0.08 ± 0.03	0.06 –
16:1ω6	1.79 ± 0.46	1.22 ± 0.30	0.59 ± 0.25	0.21 ± 0.07
16:1ω7	4.30 ± 0.92	4.53 ± 1.63	19.71 ± 2.22	8.84 ± 2.75
16:1ω8	4.12 ± 1.02	3.31 ± 0.84	– –	– –
17:1ω6	0.08 ± 0.02	0.68 ± 0.08	0.18 ± 0.08	0.18 ± 0.02
17:1ω8	0.45 ± 0.27	– –	– –	– –
18:1ω5	0.13 ± 0.04	0.11 ± 0.02	0.11 ± 0.03	0.06 ± 0.07
18:1ω6	1.63 ± 0.35	1.41 ± 0.18	1.40 ± 0.51	0.43 ± 0.07
18:1ω7	11.41 ± 0.98	12.03 ± 1.31	17.01 ± 2.81	11.90 ± 3.00
18:1ω8	tr –	1.05 ± 0.26	– –	– –
18:1ω9	4.81 ± 0.18	5.86 ± 0.25	6.12 ± 1.44	6.00 ± 1.20
19:1ω8	2.61 ± 1.91	– –	1.13 ± 1.34	1.80 ± 1.64
20:1ω5	0.06 –	– –	0.06 ± 0.02	0.04 –
20:1ω7	1.64 ± 0.04	1.94 ± 0.43	1.89 ± 0.60	1.84 ± 0.26
20:1ω8	0.07 ± 0.01	– –	– –	– –
20:1ω9	3.60 ± 0.42	3.96 ± 0.50	4.18 ± 0.47	5.00 ± 0.92
20:1ω11	3.26 ± 0.21	3.38 ± 0.24	3.41 ± 0.33	5.01 ± 0.31
18:2 a	1.05 ± 0.11	0.58 ± 0.26	0.57 ± 0.02	0.43 ± 0.12
18:2 b	0.05 –	– –	0.27 ± 0.10	0.10 ± 0.05
18:2ω6	1.62 ± 0.56	3.89 ± 1.73	4.21 ± 0.99	7.83 ± 1.42
18:3ω6	tr –	tr –	0.02 –	0.03 –
20:2 a	1.26 ± 0.42	1.31 ± 0.30	1.75 ± 0.02	0.96 ± 0.25
20:2ω7,15	3.23 ± 0.43	2.01 ± 1.10	1.77 ± 0.11	1.34 ± 0.65
20:2ω9,15	18.30 ± 2.58	13.04 ± 4.79	6.92 ± 0.91	10.58 ± 2.09
20:2 d	0.10 ± 0.01	0.95 ± 0.25	1.32 ± 0.53	0.71 ± 0.37
20:3 a	3.18 ± 0.58	2.22 ± 0.63	1.38 ± 0.31	2.66 ± 0.31
20:4ω6	6.06 ± 0.18	3.88 ± 0.27	4.54 ± 1.09	5.87 ± 0.08
20:5ω3	4.31 ± 0.32	8.08 ± 3.31	1.87 ± 0.68	6.87 ± 1.30
21:x	0.83 ± 0.26	1.09 ± 0.81	0.79 ± 0.74	1.54 ± 0.55
22:2 a	0.15 ± 0.04	– –	0.10 ± 0.03	0.09 ± 0.05
22:2ω9,15	0.33 ± 0.04	– –	0.22 ± 0.06	0.32 ± 0.07
22:3 a	0.13 ± 0.02	tr –	0.10 ± 0.02	0.10 ± 0.01
22:4ω6	1.00 ± 0.01	0.66 ± 0.19	0.45 ± 0.08	0.61 ± 0.20
22:5ω3	1.49 ± 0.09	4.21 ± 2.00	0.21 ± 0.09	0.86 ± 0.28
Total, mg g^{-1} dry wt	12.86 ± 0.43	2.80 ± 0.82	9.69 ± 1.66	3.19 ± 0.50

Fatty acid composition of neutral lipids

Fatty acid concentrations of the neutral lipids (Table 3) are higher in gills (37.5 and 28.8 mg g^{-1} dry weight for *Ifremeria nautilei* and *Alviniconcha hess-*

Table 2. Relative abundances of different groups of polar and neutral lipid fatty acids, as percentages of total fatty acids, for the symbiotic gastropods *Ifremeria nautilei* and *Alviniconcha hessleri* collected at the hydrothermal vent site Kayo (North Fiji Basin). FA: fatty acid, MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid, NMID: non-methylene-interrupted diene

Fatty acid	*Ifremeria nautilei*		*Alviniconcha hessleri*	
	Gills	Mantles	Gills	Mantles
Polar lipids				
Σ ramified FAs	1.17	3.09	0.52	0.20
Σ ω7 MUFAs[a]	17.36	18.50	38.61	22.58
Σ (ω7, ω9) MUFAs[a]	25.77	28.32	48.91	33.59
Σ (ω6, ω8) MUFAs[a]	7.61	7.00	1.99	0.64
Σ MUFAs	40.40	39.84	55.88	41.38
Σ ω3 20,22 PUFAs	5.79	12.29	2.08	7.72
Σ (ω3, ω6) 20,22 PUFAs	12.85	16.83	7.06	14.21
Σ (ω3, ω6) PUFAs	14.47	20.72	11.30	22.08
Σ NMID FAs	23.37	17.31	12.08	14.00
Σ PUFAs	43.09	41.91	26.53	40.94
Neutral lipids				
Σ ramified FAs	0.26	0.35	0.36	0.77
Σ ω7 MUFAs[a]	30.22	24.63	40.74	16.98
Σ (ω7, ω9) MUFAs[a]	37.31	35.51	47.99	28.76
Σ,(ω6, ω8) MUFAs[a]	25.07	17.45	2.03	0.81
Σ MUFAs	66.35	56.44	53.26	34.73
Σ ω3 20,22 PUFAs	1.63	5.82	2.25	7.07
Σ (ω3, ω6) 20,22 PUFAs	4.79	9.78	7.84	15.49
Σ (ω3, ω6) PUFAs	5.60	11.17	13.38	23.05
Σ NMID FAs	11.21	14.74	12.41	17.89
Σ PUFAs	19.37	30.19	28.78	47.83

[a]Even-numbered fatty acids

leri, respectively) than in mantles (12.7 and 7.7 mg g^{-1} dry weight, respectively). For both organs, concentrations were higher for *I. nautilei* than for *A. hessleri*. Ratios of neutral lipids to polar lipids (NL/PL) in gills were quite similar for both gastropods (ca 3). In mantles, these ratios were high for *I. nautilei* (4.5) and low for *A. hessleri* (2.4) The difference between the fatty acid composition of the neutral lipids and that of the polar lipids varied according to the species. In *I. nautilei* tissues, proportions of the different groups of fatty acids, such as Σ branched fatty acids, Σ MUFAs, Σ NMID or Σ PUFAs, clearly varied between polar and neutral lipids. Σ MUFAs increased at the expense of the Σ PUFAs in neutral lipids (Table 2). Particularly, the high levels of 16:1ω8 and 18:1ω7 fatty acids, especially in gills, should be noted (Table 3). In *A. hessleri* tissues, the different groups of fatty acids displayed quite similar levels in polar and neutral lipids (Table 2). However, when the neutral lipid fatty acid composition was examined in detail (Table 3), it could be observed that a strong accumulation of 16:1ω7 occurred in gills (more than a quarter of the neutral lipid fatty acids).

DISCUSSION

Indices of a food web based mainly on bacterial production

Analysis of fatty acid composition is a very useful method for studying the trophic sources used by marine organisms, whether heterotrophs (Sargent et al. 1987, 1990) or invertebrates with symbionts (Conway & McDowell Capuzzo 1990, Ben-Mlih et al. 1992, Zhukova et al. 1992, Pranal 1995, Pranal et al. in press) The fatty acid composition of *Ifreneria nautilei* and *Alviniconcha hessleri* differs greatly from that of other marine gastropods (Ackman et al. 1971, Gardner & Riley 1972, Ackman & Hooper 1973, Joseph 1982, Voogt 1983). Both hydrothermal gastropods display large amounts of MUFAs and low amounts of ω3 series PUFAs, showing that their fatty acid composition more closely resembles that of bacteria than typical marine invertebrates. As has been proposed for worms and bivalves associated with endosymbiotic bacteria (Conway & McDowell Capuzzo 1990, 1991, Giere et al. 1991, Ben-Mlih et al. 1992, Conway et al. 1992, Zhukova et al. 1992, Fullarton et al. 1995, Pranal 1995, Pranal et al. in press), this fatty acid distribution indicates a nutrition based mainly on bacterial syntheses.

Origin of the microbial markers

ω7 and ω9 series MUFAs constitute almost exclusively the total phospholipid fatty acids of aerobic bacteria (Gurr & James 1980). Particularly, palmitoleic (16:1ω7) and vaccenic (18:1ω7) acids compose most of the fatty acids of free-living sulfur-oxidizing bacteria (McCaffrey et al. 1989, Conway & McDowell Capuzzo 1990) The preponderance of these 2 markers in *Alviniconcha hessleri* tissues suggests that its nutrition is based mainly on products synthesized by sulfur-oxidizing bacteria. Endosymbionts colonizing the gills probably provide the major contribution. This assumption is supported by: (1) the presence of abundant sulfur-oxidizing bacteria in gills, indicated by the high level of palmitoleic and vaccenic acids, significantly higher in the gills (where symbionts live) than in the mantle (devoid of endobacteria), and confirmed by ultrastructural observations and RUBP carboxylase tests (authors' unpubl. results); (2) the weak diversity of the microbial markers which reflects a nutrition

based on a limited number of trophic sources; and (3) the qualitative and quantitative similarity between these microbial markers and those detected in symbiotic bivalves from deep hydrothermal vents, such as mussels and clams, known to feed mainly through sulfur-oxidizing bacteria inhabiting the gills (Ben-Mlih et al. 1992, Pranal 1995, Pranal et al. in press). The preponderance of endobacterial synthetic products in the nutrition of *A. hessleri* from the North Fiji Basin, suggested here by the fatty acid composition, is consistent with data obtained on Mariana, Manus and Lau back-arc basin specimens which indicate a sulfide-based chemoautotrophic symbiosis, as shown in TEM and by testing RUBP carboxylase activity in gills (Stein et al. 1988, Galkin 1992, Desbruyères et al. 1994), and a predominantly non-photosynthetic carbon source, as shown by analysing the carbon isotope composition (Van Dover & Fry 1989).

The situation is quite different in *Ifremeria nautilei* where the fatty acid composition differs strongly from that previously reported for heterotrophic marine gastropods (Ackman et al. 1971, Gardner & Riley 1972, Ackman & Hooper 1973, Joseph 1982, Voogt 1983) but also from that of marine invertebrates associated with sulfur-oxidizing bacteria (Conway & McDowell Capuzzo 1990, 1991, Ben-Mlih et al. 1992, Conway et al. 1992, Zhukova et al. 1992, Fullarton et al. 1995, Pranal 1995, Pranal et al. in press). *I. nautilei* tissues display high levels of 16 and 18 carbon MUFAs having the double bond in the ω6 or ω8 position. These unusual fatty acids are generally considered as markers for methane-utilizing bacteria (Ringelberg et al. 1989, Bowman et al. 1991, Guckert et al. 1991). Nevertheless, the absence of specific intracytoplasmic lamellae in the endobacterial cells, as seen in TEM, and the absence of methanol dehydrogenase activity in the gills (authors' unpubl. results) suggest that these markers originate not from endosymbionts but from the host metabolism and/or from the ingestion of free-living methanotrophic bacteria. The origin of ω6 MUFAs, which are also found in small amounts in *Alviniconcha hessleri* tissues, remains unclear (discussion below), but it seems that 16:1ω8 and 18:1ω8, only found in *I. nautilei* tissues, reflect a diet partially based on exogenously synthesized organic products. These methane-utilizing bacteria markers have never before been reported in animal tissues, making their synthesis by a gastropod unlikely. They are only detected in *I. nautilei*, where they constitute more than 4% of the to-

Table 3. Fatty acid composition of the neutral lipids, as a percentage of total fatty acids, from the symbiotic gastropods *Ifremeria nautilei* and *Alviniconcha hessleri* collected at the hydrothermal vent site Kayo (North Fiji Basin). Values represent the mean of 2 different animals ± SD. NL/PL: ratio of neutral lipid fatty acid / phospholipid fatty acid concentration; tr: trace

Fatty acid	*Ifremeria nautilei*		*Alviniconcha hessleri*	
	Gills	Mantles	Gills	Mantles
14:0	0.99 ± 0.32	0.69 ± 0.07	0.59 ± 0.05	0.49 ± 0.08
15:0	0.08 ± 0.01	– –	0.27 ± 0.05	tr –
16:0	8.38 ± 0.64	8.16 ± 0.44	13.13 ± 1.83	8.04 ± 3.27
17:0	0.32 ± 0.04	0.25 ± 0.11	0.31 ± 0.10	0.44 ± 0.19
18:0	4.73 ± 0.30	5.50 ± 0.52	3.54 ± 0.89	9.32 ± 2.58
a 15:0	0.05 ± 0.01	– –	– –	– –
i 17:0	– –	– –	0.08 ± 0.01	– –
a 17:0	0.08 ± 0.02	0.16 ± 0.02	0.08 ± 0.03	– –
i 19:0	– –	0.09 ± 0.03	0.21 ± 0.10	0.58 ± 0.15
a 19:0	0.12 ± 0.03	0.09 ± 0.04	– –	0.20 ± 0.01
16:1ω5	0.63 ± 0.17	0.42 ± 0.18	0.08 ± 0.03	– –
16:1ω6	3.76 ± 0.48	2.36 ± 0.06	0.81 ± 0.31	0.18 ± 0.02
16:1ω7	8.35 ± 1.23	5.80 ± 0.12	27.49 ± 1.92	4.31 ± 1.41
16:1ω8	12.29 ± 2.07	7.26 ± 0.14	– –	– –
17:1ω6	0.22 ± 0.01	– –	0.05 ± 0.01	– –
17:1ω8	0.13 ± 0.04	– –	0.06 –	– –
18:1ω5	0.30 ± 0.07	0.30 ± 0.07	0.06 ± 0.01	– –
18:1ω6	4.67 ± 0.57	4.94 ± 1.99	1.22 ± 0.17	0.63 ± 0.21
18:1ω7	17.75 ± 0.56	15.70 ± 3.25	11.53 ± 0.73	9.70 ± 3.14
18:1ω8	3.85 ± 0.34	2.59 ± 0.45	– –	– –
18:1ω9	3.04 ± 0.06	4.75 ± 2.19	3.24 ± 0.36	3.61 ± 0.14
19:1ω8	0.25 ± 0.03	0.45 ± 0.36	0.61 ± 0.24	2.04 ± 1.24
20:1ω5	0.16 ± 0.03	– –	0.08 ± 0.03	– –
20:1ω7	4.12 ± 0.62	3.13 ± 0.56	1.71 ± 0.07	2.97 ± 0.97
20:1ω8	0.50 ± 0.08	0.30 ± 0.19	– –	– –
20:1ω9	4.05 ± 0.57	6.13 ± 1.09	4.01 ± 0.13	8.17 ± 1.77
20:1ω11	2.15 ± 0.49	2.13 ± 0.54	2.30 ± 0.59	3.13 ± 0.46
18:2 a	0.60 ± 0.19	0.51 ± 0.23	0.42 ± 0.02	0.21 ± 0.12
18:2 b	0.06 ± 0.02	– –	0.19 ± 0.07	– –
18:2ω6	0.81 ± 0.09	1.39 ± 0.71	5.54 ± 0.57	7.56 ± 2.62
18:3ω6	– –	– –	– –	– –
20:2 a	0.66 ± 0.20	0.84 ± 0.38	1.39 ± 0.08	0.89 ± 0.07
20:2ω7,15	1.96 ± 0.49	1.96 ± 0.24	2.57 ± 0.16	3.66 ± 1.83
20:2ω9,15	7.78 ± 1.60	10.78 ± 2.63	6.85 ± 0.78	11.08 ± 0.53
20:2 d	0.28 ± 0.09	0.70 ± 0.21	1.40 ± 0.26	0.77 ± 0.11
20:3 a	1.24 ± 0.16	1.61 ± 0.31	1.24 ± 0.34	3.24 ± 0.09
20:4ω6	2.74 ± 0.84	3.48 ± 1.32	5.27 ± 0.99	7.67 ± 0.66
20:5ω3	1.16 ± 0.15	4.44 ± 3.45	1.98 ± 0.56	6.20 ± 1.19
21:x	0.49 ± 0.08	2.09 ± 1.72	1.05 ± 0.33	3.26 ± 1.58
22:2 a	0.10 ± 0.01	0.09 ± 0.02	0.08 ± 0.02	0.46 ± 0.03
22:2ω7,15	0.43 ± 0.03	0.37 ± 0.09	0.12 ± 0.04	1.04 ± 0.09
22:3 a	0.17 ± 0.02	0.07 ± 0.02	0.08 ± 0.02	0.18 ± 0.02
22:4ω6	0.41 ± 0.16	0.48 ± 0.24	0.31 ± 0.06	0.74 ± 0.09
22:5ω3	0.47 ± 0.07	1.38 ± 1.12	0.27 ± 0.02	0.88 ± 0.38
Total, mg g^{-1} dry wt	37.46 ± 8.20	12.67 ± 5.74	28.77 ± 3.85	7.65 ± 1.27
NL/PL	2.91 ± 0.37	4.53 ± 1.69	2.97 ± 0.45	2.40 ± 0.39

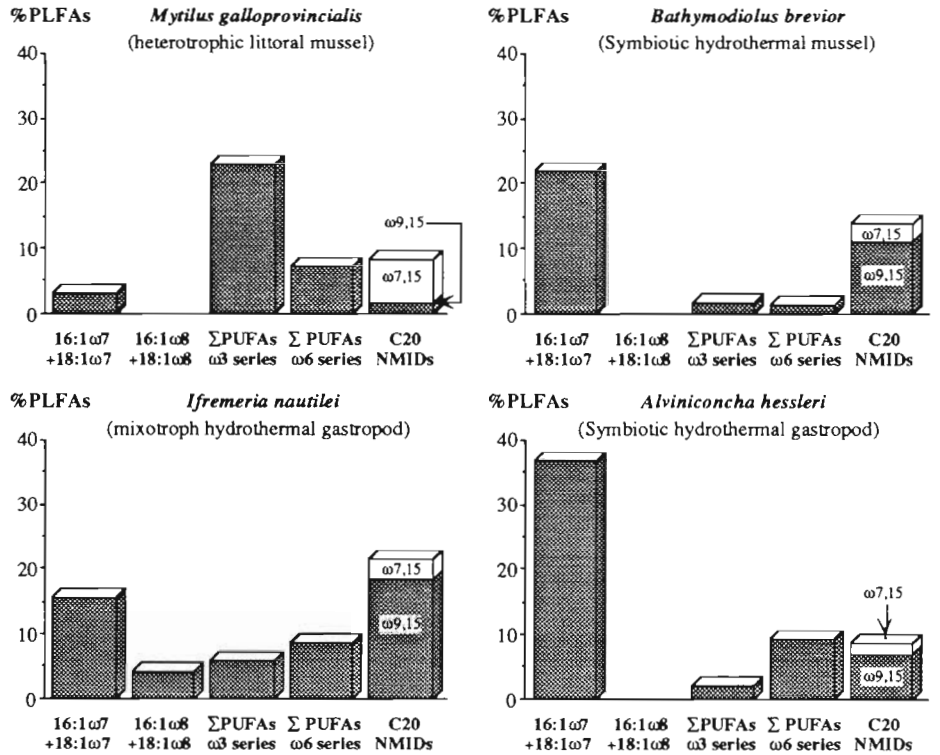

Fig. 1. Proportions of different groups of fatty acids, as percentages of total fatty acid phospholipids (PLFAs), in gills of the littoral mussel, *Mytilus galloprovincialis*, from Banyuls (West Mediterranean) and the hydrothermal molluscs, *Bathymodiolus brevior* (Pranal et al. in press), *Ifremeria nautilei* and *Alviniconcha hessleri*, from site Kayo (North Fiji Basin, West Pacific). PUFAs: polyunsaturated fatty acids; NMIDs: non-methylene-interrupted dienes

tal phospholipid fatty acids, and not in *A. hessleri*, which is believed to satisfy its requirements from the endosymbionts. Thus they probably originate from the ingestion of free-living methanotrophic bacteria. As compared to *A. hessleri*, the hypothesis of a partially heterotrophic nutrition for *I. nautilei* is supported by: (1) the diversity and the relatively large amounts of fatty acid markers specific to various bacterial types, consisting mainly of branched and monounsaturated odd-numbered fatty acids generally related to anaerobic bacteria (Parkes & Taylor 1983, Sargent et al. 1987, Rajendran et al. 1994), in vaccenic acid found in large amounts in aerobic micro-organisms such as sulfur-oxidizing bacteria (McCaffrey et al. 1989, Conway & McDowell Capuzzo 1990) and of ω8 series MUFAs specific to methanotrophs; (2) the homogeneous distribution of the microbial markers among organs, which could correspond to a dietary assimilation of bacterial products followed by their similar distribution between organs rather than the input of endobacterial fatty acids produced solely in the gills; and (3) the relatively high level of plant-derived fatty acids, particularly with respect to ω3 series PUFAs (Fig. 1).

Thus, according to the discrepancies between the nature and the distribution of the microbial markers detected in *Alviniconcha hessleri* and *Ifremeria nautilei* tissues, it seems that these 2 gastropods display distinct nutritional strategies, that for the former being mainly based on the sulfur-oxidizing symbiont production and that for the latter being probably mixotrophic, with a contribution from sulfur-oxidizing symbiont production as well as from grazed bacterial mats partially composed of free-living methane-utilizing bacteria. Our results are in agreement with *in situ* observations indicating a specific distribution of both gastropods on the same site, *I. nautilei* being not so strictly associated with the vent opening as *A. hessleri* (Galkin 1992, Desbruyères et al. 1994). This specific distribution could be related to the distribution of the 2 main hydrothermal energy sources for bacterial chemosyntheses, hydrogen sulfide and methane. Because of its rapid oxidation in sea water, hydrogen sulfide has a more localized distribution in the hydrothermal vent area than methane (Gal'chenko 1988). Thus, differentiation in the spatial distribution of these 2 gastropod species at any one site is probably related to their specific nutritional strategy, *A. hessleri* being closely associated with the vents in order to fuel the sulfur-oxidizing endosymbionts and *I. nautilei* being more peripherally distributed, in areas where it can both provide its

endosymbionts with sulphides and graze bacterial mats, among which there may be methanotrophs. Nevertheless, while the trophic needs of both hydrothermal gastropods appear to be largely satisfied by the microbial products synthesized *in situ*, distinct nutritional strategies may allow these sympatric gastropods to avoid direct competition for their energy supply.

Hydrothermal gastropod metabolism

Compared with the symbiotic mussels collected at the same site, hydrothermal gastropods display conspicuous levels of ω6 PUFAs, on the same order of magnitude as that of a littoral heterotrophic mussel (Fig. 1). This is all the more noticeable for *Alviniconcha hessleri* as this gastropod is supposed to derive energy mainly from products synthesized by endosymbionts, which is confirmed by the low level of ω3 PUFAs (on the same order of magnitude as that of the symbiotic mussels), particularly in gills. It is generally accepted that animals desaturate fatty acids using Δ9 desaturase, 16:0 and 18:0 being at the base of desaturations and further elongations occurring via 16:1ω7 and 18:1ω9 synthesis (Gurr & James 1980). In contrast, plants are capable of Δ12 and Δ15 desaturations and thus constitute the main source of ω3 and ω6 PUFAs which are considered to be essential for animals (Gurr & James 1980, Sargent et al. 1987, 1990). Nevertheless, several lines of evidence indicate that some terrestrial gastropods are capable of *de novo* biosynthesis of linoleic acid, 18:2ω6, from acetate (Van Der Horst 1973, Van Der Horst & Oudejans 1976, Weinert et al. 1993). Although the enzyme responsible for this peculiar desaturation has not been investigated, Weinert et al. (1993) supposed that these terrestrial gastropods use the same pathway as plants and some insects, based on a Δ12 desaturation of oleic acid (Cripps et al. 1990, Borgeson et al. 1991). Compared to other marine heterotrophic invertebrates, such as bivalve molluscs or crustaceans (Ackman et al. 1971, Ackman & Hooper 1973, Dall et al. 1991) or symbiotic mussels from the same hydrothermal site (Pranal 1995, Pranal et al. in press), *A. hessleri* and *Ifremeria nautilei* display particularly high levels of linoleic acid in their tissues. As in terrestrial gastropods, linoleic acid could originate from the host metabolism via Δ9 desaturation of stearic acid, yielding 18:1ω9, which should be Δ12 desaturated to produce 18:2ω6. However, considering the presence of low amounts of 16:1ω6 and 18:1ω6 in tissues of both gastropods, linoleic acid could be synthezised from Δ10 desaturation of palmitic acid, yielding 16:1ω6, which should then be chain-elongated to 18:1ω6 and then Δ9-desaturated to produce 18:2ω6. In both cases, linoleic acid could originate from the host metabolism and ω6 series PUFAs would not be considered as essential fatty acids for the hydrothermal gastropods.

ω6 PUFAs may also originate from the ingestion of free-living microbes. This dietary input could be indirect via the desaturation and/or the elongation of 16:1ω6 and 18:1ω6 fatty acids assimilated by grazing methane-utilizing bacterial mats. Concerning *Alviniconcha hessleri*, the absence of other markers of methanotrophic bacteria (e.g. ω8 series MUFAs) makes this assumption questionable. A further possibility is a direct dietary input of ω6 PUFAs from the ingestion of benthic heterotrophic microbes such as prokaryotes which are able to synthesize their own long-chain PUFAs, as they have been previously reported to occur in deep-sea environments (Delong & Yayanos 1986), or microeukaryotes enriched with ω6 PUFAs. Comparing the lipid composition of 2 littoral benthic invertebrates, one being a filter-feeder and the other a deposit-feeder, Bell & Sargent (1985) observed that the deposit-feeder displayed a higher level of ω6 PUFAs than the filter-feeder. They hypothesized that this difference results from the ingestion of benthic microeukaryotes by the deposit-feeder. The same could be hypothesized in hydrothermal environments where symbiotic gastropods, which possess potentially functional organs for grazing, have a noticeably higher level of ω6 PUFAs than symbiotic mussels (Fig. 1), which are known to possess potentially functional organs for filter-feeding.

Whatever the origin of ω6 series fatty acids, hydrothermal gastropods do not appear to be depleted of ω6 series PUFAs. The level of these essential fatty acids in both deep-sea gastropods is more similar to that of heterotrophic littoral molluscs than that of symbiotic mussels collected at the same site (Fig. 1). The hydrothermal gastropods probably use an alternative pathway, based on endogenous or free-living bacterial products rather than phytoplanktonic ones,—as is supposed for most marine heterotrophic invertebrates—to maintain their specific fatty acid composition.

In heterotrophic marine invertebrates, some specific fatty acids such as non-methylene-interrupted dienes (NMIDs) have been supposed to be acceptable as substitutes for ω3 PUFAs (Ackman & Hooper 1973, Klingensmith 1982). In bivalves known to feed mainly through their associated chemoautotrophic bacteria, the inverse relationship between the level of NMIDs and that of PUFAs considered to be essential reinforces this supposition (Berg et al. 1985, Zhukova et al. 1992, Fang et al. 1993). Like symbiotic bivalves, hydrothermal gastropods display substantial amounts of NMIDs. These dienoic fatty acids could be related to a depletion of ω3 PUFAs. In particular, 20:2ω9,15 is conspicuously predominant. It is of interest to note that the

same compound is the major NMID constituent detected in all deep-sea symbiotic bivalves analysed until now: methanotrophic (Fang et al. 1993) and sulfur-oxidizing mytilids (Pranal 1995, Pranal et al. in press) as well as sulfur-oxidizing vesicomyids (Pranal 1995). In contrast, NMIDs in littoral symbiotic bivalves appear to consist mainly of 22:2ω7,15 (Berg et al. 1985, Zhukova et al. 1992, Fullarton et al. 1995, Pranal 1995). The ubiquity of 20:2ω9,15 in deep-sea symbiotic molluscs strongly suggests a functional significance of this fatty acid in relation with depth. It is admitted that the chain length as well as the position of the unsaturations modify the physical properties of the fatty acids. For instance, Lomascolo et al. (1994) noted that maintenance of membrane fluidity at low temperature required the accumulation of short-chain fatty acid phospholipids and Barton & Gunstone (1975) showed that, among several PUFA isomers, the lowest melting point was found in those which retain double bonds in the more central position. Consequently, synthesis of NMIDs by molluscs mainly feeding through endobacterial production appears to be a means to restore the depleted essential ω3 series PUFAs. More particularly, in deep-sea symbiotic species, the favoured synthesis of 20:2ω9,15 compared to 22:2ω7,15 probably maintains the membrane fluidity and thus could be considered an adaptation to the low temperature and the high hydrostatic pressure characterising the deep-sea environment.

Energy storage

In animals, neutral lipids are essentially used for energy storage (Gurr & James 1980, Voogt 1983). In contrast to the phospholipid fatty acids, this lipid class does not display a specific composition but depends on the dietary inputs (Joseph 1982, Voogt 1983). Neutral lipids of both studied gastropods are characterized by an accumulation of bacteria-derivated fatty acids, especially in gills. This accumulation confirms the preponderance of the bacterial input in the nutrition of the hydrothermal gastropods. In *Alviniconcha hessleri*, the increase of putative microbial markers is reflected essentially by C16:1ω7 and is probably related to the assimilation of an excess of palmitoleic acid originating from the sulfur-oxidizing endobacteria. In *Ifremeria nautilei*, most of the MUFAs occur at high levels, particularly those of the ω6, ω7 and ω8 series. As also proposed for phospholipids, the diversity of the microbial markers is probably related to both carbon transfer from the endobacteria to the host and assimilation from the ingestion of free-living bacteria. Furthermore, in this species the high level of 16:1ω8 in neutral lipids could indicate the inability to use this unusual fatty acid for physiological or structural purposes and thus its accumulation in neutral lipids for energetic needs. Indeed, fatty acids used as fuel do not require a peculiar configuration.

Compared to heterotrophic gastropods from littoral areas (Ackman et al. 1971, Dall et al. 1991) and to symbiotic mussels collected at the hydrothermal site (Pranal et al. in press), the hydrothermal gastropods display high concentrations of neutral lipids, particularly in gills. Despite the preferential use of glycogen by typical molluscs (Bayne et al. 1976, Livingstone & De Zwaan 1983), the remarkable concentrations of neutral lipids found in *Ifremeria nautilei* and in *Alviniconcha hessleri* tissues suggest a preferential use of these compounds. Neutral lipids are the most condensed form of energy storage (Gabbott 1976, Gurr & James 1980). Their accumulation in gills of both provannid gastropods, probably resulting from the degradation of bacterial phospholipids, could be considered as an adaptation to temporally unstable and spatially discontinuous environments, permitting the hydrothermal gastropods to migrate to other sites when the activity of a site is declining, as inferred from ecological observations (Denis et al. 1993).

Efficacy of some fatty acids for describing the nutrition of marine invertebrates

Comparative analysis of the fatty acid composition from various marine invertebrates shows that a diet based mainly on bacterially synthesized products is characterised by a drastic enrichment of MUFAs and a concomitant decrease of PUFAs. Particularly in the case of bacterial symbiosis, palmitoleic (16:1ω7) and vaccenic (18:1ω7) acids appear to be ubiquitous markers for sulfur-oxidizing endobacteria. Thus, in phospholipids, their predominance compared to the other fatty acids can be directly related to the presence of sulfur-oxidizing symbionts. Furthermore, high levels of these markers in lipids other than phospholipids, particularly in neutral lipids, and in tissues other than those hosting the endobacteria indicate an important transfer of the endobacterial products to the host. In most of the marine symbiotic invertebrates studied, the dominant role of the symbionts in the host's nutrition is closely related to the decrease of the plant-derived fatty acid level (mostly ω3 and ω6 series PUFAs). Nevertheless, fatty acid analysis of symbiotic gastropods shows that ω6 PUFAs are equivocal markers which do not allow a clear estimate of the proportion of exogenous inputs in the symbiotic invertebrate diet. Only ω3 PUFAs can unambiguously define the heterotrophic part of the diet. For symbiotic invertebrates from deep-sea hydrothermal environments, in addition to the ω3

PUFAs which reflect the heterotrophic inputs based on processes outside the sites (i.e. photosynthetic production from the photic zone), some markers such as ω8 MUFAs allow estimation of the heterotrophic inputs originating from the sites (i.e. chemosynthetic production). Marine molluscs can use a number of nutritional strategies which vary, according to the species, from entirely heterotrophic to essentially symbiotic inputs.

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